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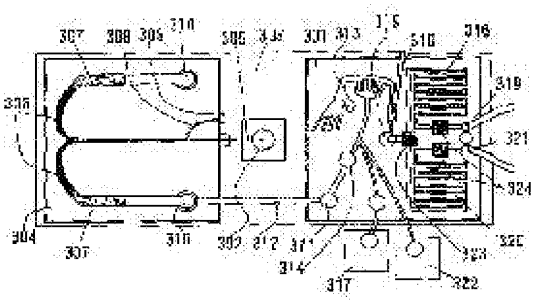
(54) METHOD OF ANALYZING BLOOD, APPARATUS FOR ANALYZING BLOOD, AND METHOD OF MANUFACTURING APPARATUS FOR ANALYZING BLOOD

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method of manufacturing a chip using a disposable, low-priced substrate, a method of correcting a measurement error caused by a time-varying change in a detecting system by mixing blood plasma or blood serum with a buffered liquid substrate or coloring liquid in a microchip, and a method of confining illuminating light completely within a micro flow channel without leakage into the outside.
SOLUTION: A painless needle for blood collection on the chip, a U-shaped capillary for isolating blood cells, blood plasma, or blood serum, the micro flow channel for blood plasma or blood serum and a reagent, a mixer using centrifugal force, and an optical path for light are arranged. Using a small amount of blood, the concentrations of γ-GTP. GOT, and GOT can be measured in a short time. For the miniaturization and price-reduction of the apparatus, a cell inlet, the flow channel, a branched flow channel, and a pumping means are integrally on the chip, and as the pumping means, an electroosmatic flow pump is used which does not directly apply an electric field to cells.



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* NOTICES *

CLAIMS

[Claim(s)]
[Claim 1]
From a means to extract blood, a filtration means to filter the blood concerned extracted at least and to obtain plasma or a blood serum, or the blood concerned, among means to separate plasma or a blood serum from in the living body in one or more substrates One of means, A means to mix the dyadic eye with plasma or a blood serum concerned, and the substrate buffer solution for a color coupler at least, Hemanalysis equipment characterized by having a measurement means for the transmittance of the exposure light which equips with a light irradiation device a means to move plasma or a blood serum to the mixed means concerned with a pump, a means to move the mixed liquor concerned to the passage for measurement, and the end of the passage for measurement, and equips other ends with a photodetector.
[Claim 2]
Hemanalysis equipment according to claim 1 is hemanalysis equipment characterized by measuring the concentration of three enzymes of |-glutamyl transpeptidase, and GOT and GPT which serve as a marker of a diagnosis of a liver function.
[Claim 3]
Especially a means to separate plasma or a blood serum according to claim 1 is hemanalysis equipment characterized by consisting of passage of a W character mold.
[Claim 4]
Hemanalysis equipment with which the cross section of the passage of the middle where blood is introduced first is characterized by being smaller than that of the passage of both sides in W mold passage according to claim 3.
[Claim 5]
Hemanalysis equipment characterized by constituting the bypass passage which the passage between a means to extract blood according to claim 1, and a filtration means or a means to

dissociate branches, and passes out of hemanalysis equipment.
[Claim 6]
Hemanalysis equipment characterized by the substrate which constitutes hemanalysis equipment according to claim 1 consisting of polymer resin.
[Claim 7]
Hemanalysis equipment with which polymer resin according to claim 6 is especially characterized by being optically opaque.
[Claim 8]
A means according to claim 1 to mix is the mixed approach characterized by mixing to homogeneity by moving in the inside of hemanalysis equipment and making a mixed liquor-ed object stir according to a centrifugal force.
[Claim 9]
Especially the means to which blood according to claim 1 is moved is hemanalysis equipment by which it is being [it / the electroendosmose style pump constituted in hemanalysis equipment] characterized.
[Claim 10]
Especially the means to which blood according to claim 1 is moved is hemanalysis equipment by which it is being [it / a pump besides hemanalysis equipment] characterized.
[Claim 11]
The passage for gamma-GTO measurement given in claims 1 and 2 is hemanalysis equipment characterized by consisting of passage for spectrometry of two or more same configurations especially.
[Claim 12]
A part of front face [at least] of the passage for measurement according to claim 1 is hemanalysis equipment characterized by being covered with the ingredient which uses Teflon as a principal component.
[Claim 13]
A part of front face [at least] of the passage for measurement according to claim 1 is hemanalysis equipment characterized by being covered with the ingredient which uses a metal as a principal component.
[Claim 14]
Hemanalysis equipment characterized by including the field where a normal vector becomes about 45 degrees to the travelling direction of a measuring beam in at least one place of the side attachment wall which constitutes the passage for measurement according to claim 1.
[Claim 15]
Hemanalysis equipment according to claim 1 is the hemanalysis approach characterized by controlling the temperature of the hemanalysis equipment concerned on a centrifugal separator.
[Claim 16]
It is the hemanalysis approach characterized by contacting a Peltier device to the hemanalysis equipment concerned, and performing temperature of hemanalysis equipment according to claim 15, as for control.
[Claim 17]
Hemanalysis equipment according to claim 1 is the hemanalysis approach characterized by performing component analysis in blood in the passage for measurement after separating and obtaining plasma or a blood serum from blood on a centrifugal separator and mixing plasma or a blood serum concerned with various reagents on the centrifugal separator concerned succeedingly.
[Claim 18]
The hemanalysis approach characterized by making into a reduced pressure ambient atmosphere some [which extracts the skin blood collecting part concerned and blood / at least] perimeters of a means when contacting a means to extract the blood of hemanalysis equipment according to claim 1 on the skin like a doner site-ed.
[Claim 19]
Blood extraction equipment which has the device in_which hemanalysis equipment can be moved

in the direction into which a part of means [at least] which installs hemanalysis equipment, and has a connecting means with the pump for considering the inside of the tub which isolates a part of means [at least] which extracts the blood for maintaining a reduced pressure ambient atmosphere according to claim 18 with the external world, and the tub concerned as reduced pressure, and extracts blood advances into the skin.

[Translation done.]

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]
This invention extracts blood from an aponia needle without a pain, and measures with an absorbance the reduction rate of the resultant concentration produced within the substrate buffer solution which measured or mixed the absorbance of the coloring matter produced from substrate buffer-solution **** and the mixed liquor which added coloring liquid further with the plasma or the blood serum which obtained by performing centrifugal separation, that is, relates to the equipment and the manufacture approach of measuring a liver function with a colorimetric method. Especially, these at least one function is accumulated on the chip substrate of one at least, the chip substrate is still smaller, and the knowledge of special medicine and rating do not need for the handling, but it is related with the liver function diagnostic device characterized by performing an above-mentioned liver function diagnosis for a short time simply.
[0002]
[Description of the Prior Art]
The liver function of ****** is diagnosed by measuring the value of three enzymes, GPT (Glutamic-pyruvic transaminase), GOT (Glutamic-oxaloacetic transaminase), and |-glutamyl transpeptidase (gamma-Glutamyltranspeptid ase). Many these three enzymes especially to liver exist in in the living body, and the enzyme activity value in a blood serum is usually kept constant. For example, as for 8 - 48IU / liter, and GPT, the range of GOT of 13 - 35IU / liter, and |-glutamyl transpeptidase:7 - 60IU / liter is the normal-values range of an adult. IU shows International Unit here. However, if a hepatopathy etc. is caused, it is known that these activity values in a blood serum will rise. Therefore, it is indispensable to the decision of a treatment policy, and observation of therapy progress in a liver function diagnosis and a list to measure three markers.
[0003]
Inspection of these three markers is conducted by automatic hemanalysis inspection with many of other analysis items. A general-purpose automatic hemanalysis inspection is large-scale and expensive equipment which consists of a sample preparative isolation measuring system, a reagent preparative isolation measuring system, the system of reaction, the detection system of measurement based on a colorimetric method, a washing system, and control and a data-processing system using a lot of blood of the milliliter which collected blood with the syringe.

And the present condition is that inspection of only a liver function also cannot but use this equipment, and long duration is taken for an inspection result to come out moreover.
[0004]
On the other hand, various minute passage and microprobe analysis machines of a drug solution are arranged on the monotonous substrate of several cm around which are said to a foundation as recent years, mu-TAS (MicroTotal Analytical System), and Lab.ona Chip (research facility on a chip) in an advance of the ultra-fine processing technology accompanying a semi-conductor product, and the attempt which performs various chemosynthesis and physical chemistry analysis on the substrate is performed briskly. The electric energy which needs neither large space nor a large-scale and expensive measuring device like the so-called conventional chemistry laboratory as an advantage which performs the aforementioned chemosynthesis and analysis on such the point of a finger and the chip of this dimension, but is needed for that the chip itself is portability, and the amount of drug solutions to be used and analysis decreases remarkably, and it is mentioned that it is environment-friendly etc. It introduces into the micro passage which has the width of face of dozens - 100 micrometers of numbers and the depth on the chip by the blood of the ultralow volume extracted from the aponia needle as one big application of such a chip. The health care chip (application for patent 2001-258868) which separates a corpuscle from blood on the chip concerned, and measures a health marker from the obtained plasma, A chip, a protein chip, etc. which separate DNA at high speed are briskly studied and developed as a foundation of the minute hydrodynamics (microfluidics) based on a fluid moving in super-thin passage.
[0005]
Once, as business, if the blood of the ultralow volume of this mu-TAS extracted from the aponia needle to the substrate of one small-scale method is introduced into the micro passage on a chip and the microchip in which a liver function diagnosis is possible is realized from that plasma or a blood serum It not only brings about a suitable medical examination, but can inspect a patient quickly at a bedside, a patient's condition can grasp by the doctor in a short time, and it sets to being home. The health care to alcoholic intake, the class of meal, the need for a sport, etc. is made possible, and it is expected that it is greatly further useful also to a diagnosis of condition of disease. Moreover, this invention using a colorimetric method can be applied also to measurement of the marker of lifestyle-related diseases, such as a glucose, a creatinine, and an urea nitrogen, and can be developed also to the detector which diagnoses the urgent many items in the health care chip and bedside for health care in being home.
[0006]
[Problem(s) to be Solved by the Invention]
In order to measure a liver function simple and quickly using a microchip, a chip must be produced using a substrate like the cheap polymer which can be thrown away first. Moreover, high sensitivity and in order to measure with high precision, by measurement of gamma-glutamyl transpeptidase, the substrate buffer solution and coloring liquid must be mixed for three markers of GPT, GOT, and |-glutamyl transpeptidase into plasma or a blood serum. GOT measurement also mixes the substrate buffer solution with GPT into plasma or a blood serum, and the reduction rate of the concentration of the produced resultant is measured with an absorbance. Therefore, in the mensuration concerned, mixing of a solution is indispensable. However, since as for the solution in the inside of the micro passage of mum dimension viscosity becomes large from the convection current, namely, the Reynolds number becomes low, as for the flow of the solution in the inside of micro passage, a laminar flow becomes dominant. So, even if it introduces two or more liquid into micro passage, it flows uniquely [without mixing each] and mixing is very difficult. Moreover, in measurement of gamma-glutamyl transpeptidase, in case three markers are measured with a colorimetric method, in order to measure the concentration of the coloring matter produced from the mixed liquor which added coloring liquid, a measurement error arises by fluctuation of the light source, and aging of the detection system containing a photodetector and substrate temperature etc. In GPT and GOT measurement, the concentration of a resultant is boiled in micro passage and the spectrometry of the light of the wavelength corresponding to the energy of each chemical bond is called for. In order to measure

these absorbances by the high S/N ratio, light passes the longest possible distance in micro passage, and it is made to absorb with coloring matter and a resultant concerned, and if it is ****, there is nothing. For that purpose, it is required that exposure light should carry out total reflection with the wall of micro passage.

[0007]

The purpose of this invention is to offer the approach of mixing the substrate buffer solution and coloring liquid with the production approach of a chip into plasma or a blood serum within a microchip, and amending the measurement error by aging of a detection system etc. using the cheap substrate which can be thrown away, and the approach of shutting up in passage altogether, without exposure light leaking to the exterior of micro passage.

[0008]

[The means for making a technical problem solved]

Into the plasma or the blood serum which extracted blood, poured into W character-like passage, and obtained by performing centrifugal separation in the passage from the aponia needle without a pain, in the case of |-glutamyl transpeptidase Coloring material is mixed with the substrate buffer solution, and it mixes using a centrifugal force, and the absorbance of the coloring matter produced from the mixed liquor concerned is measured, and it is. In the case of GTP or GOT In order to measure with an absorbance the reduction rate of the resultant concentration produced within the substrate buffer solution mixed using the centrifugal force and to promote absorption by the coloring matter of incident light in that case, The both ends of passage give the include angle of 45 degrees so that incident light may go into passage efficiently and may carry out outgoing radiation, and since the optical path length is lengthened further, passage can bend. In a field The side attachment wall of passage produces a chip using the cheap substrate which measures a liver function and which can be thrown away to high degree of accuracy and high sensitivity by making it the include angle of 45 degrees, carrying out the coat of Teflon membrane etc. to the wall of passage, and carrying out total reflection.

[Embodiment of the Invention]

[0009]

First, the measurement principle of |-glutamyl transpeptidase, and GOT and GPT is explained. When incidence of the light of reinforcement I0 is carried out to the solution of concentration c and, as for these, luminous intensity decreases die-length L to I after transparency, t is set to I/I0=10-k0L by transmittance, and the optical path length L is set to absorbance [of E-1n (I/I0)] =k0c (c is a constant) at the fixed time. This is called principle of Beer. In measurement of |-glutamyl transpeptidase, the active mass of |-glutamyl transpeptidase is calculated by carrying out the quantum of the coloring matter generated by operation of a |-glutamyl transpeptidase enzyme. In actual measurement, if the |-glutamyl transpeptidase enzyme in plasma acts on the mixed liquor which added plasma or a blood serum to the substrate buffer solution of a L-gamma-glutamyl-p-N-ethyl-N-hydroxyethylamino anilide and a glycylglycine called HA substrate method, for example, a p-N-ethyl-N-hydroxyethylamino aniline will generate. The 1-naphthol-2-sulfonic acid in this and the substrate buffer solution reacts, and further, periodic acid works as an oxidizer and generates blue coloring matter. This shows the absorption spectrum which has a peak in 660nm, irradiates 660nm light, and measures the absorbance E (bibliography; the Fujisawa **, other:Japan clinical (spring first issue) 38,889-895).

[0010]

The GOT enzyme in plasma will act and the measurement principle of GOT will generate glutamic acid and oxaloacetic acid, if plasma or a blood serum is added to the substrate buffer solution containing L-asparatic acid and alpha-ketoglutarate. The generated oxaloacetic acid changes with operations of a malate dehydrogenase (MDH) to a malic acid under existence of a beta-nicotinamide adeninedinucleotide reduction type (NADH). At this time, NADH oxidizes to a beta-nicotinamide adeninedinucleotide oxidation type (NAD), and the absorbance of 340nm decreases. The GOT activity value in plasma is calculated by measuring this reduction rate. The GPT enzyme in a sample will act and the measurement principle of GPT will generate glutamic acid and a pyruvic acid, if plasma is added to the substrate buffer solution containing L-alanine and alpha-ketoglutarate. The generated pyruvic acid changes with operations of lactate

dehydrogenase (LDH) to a lactic acid under existence of a beta-nicotinamide adeninedinucleotide reduction type (NADH). At this time, NADH oxidizes to a beta-nicotinamide adeninedinucleotide oxidation type (NAD), and the absorbance of 340nm decreases. The GOT activity value in plasma is calculated by measuring this reduction rate (bibliography; Masayuki Saito, Shoji Tamba (piece), "clinical chemistry", Kodansha 116 (1981)).

[0011]

In order to **** more this invention which measures |-glutamyl transpeptidase and three markers of GPT and GOT by the microchip in a detail based on an above-mentioned measurement principle, this is explained along with an attached drawing.

[0012]

Fig. 1 stabs people's skin with an aponia needle, extracts blood, and shows the schematic diagram of the equipment poured into the chip for corpuscle separation. (101) -- an aponia needle -- it is -- the tip of stain loess tubing of the diameter of about 90 micrometer -- 10 degrees -- also grinding -- it is what was further made sharp by electrolytic polishing, and since it is thin, a pain is not sensed almost. While achieving the duty softened even if (102) is cylinder tubing, contacts the skin first and has a pain from the feeling of contact, blood is drawn in a chip with an external pump from a suction jig [having been prepared in the corpuscle separation chip of (103) (104)], a vessel is interlocked with, the skin front face in contact with cylinder tubing is made reduced pressure, the skin is risen, and a needle is automatically stuck in the skin. instead of [of this external pump] -- small [like an electroendosmose style pump] -- and -- high -- you may use it, preparing in pump hemanalysis equipment and one. [****] (105) is a pipe connected with an external pump. (106) shows the jig with which (107) performs W character-like passage of a chip and an electrode holder and (108) perform insertion of a corpuscle separation chip and ejection. (109) shows a bypass. In case the reason for having prepared this separates a corpuscle with a centrifugal force on the below-mentioned chip (it is called on-chip corpuscle separation), it is because the blood which has collected in the aponia needle coagulates in centrifugal separation, and is got blocked within a needle and it becomes impossible to pull out the obtained plasma with a pump outside from the inside of a chip. During blood collecting, the edge of this bypass forced a thing like sponge, with the ambient atmosphere of the hemanalysis equipment exterior, it was intercepted by ******, and confidentiality has been obtained. Moreover, why the passage of the chip for corpuscle separation of (106) has become W character-like is explained below. That is, although a corpuscle collects after centrifugal separation at the pars basilaris ossis occipitalis of U character-like passage and plasma is obtained in the passage of both sides in U character-like passage, the plasma volume obtained only at one side is restricted. On the other hand, much plasma will be obtained by the passage of both sides, if centrifugal separation is performed after the shape of W character shared the first-class U character-like way, makes it thin, and introduces whole blood from an aponia needle and whole blood is filled in the thick passage of both sides. Moreover, although this corpuscle separation chip is manufactured with a polymer substrate like PET (polyethylene terephthalate), shortly after whole blood is introduced into the passage made from PET, an erythrocyte and protein adhere to the wall of passage and coagulation also takes place further. In order to prevent it, the coat of the MPC (2-methacryloyloxyethylphosphorylcholine; bibliography KIshihara, H.Oshida, T.Ueda, Y.Endo, A.Watanabe and N.Nakabayashi:J.Biomat.Mat.Res:26(1992) 1543.) polymer is carried out to a wall. In addition, although corpuscle separation carried out description which performed centrifugal separation on chip, things can do the detailed pillar from which the dimension shown in the application for patent 2001-258868, of course differs using the filtration means established in passage.

[0013]

Fig. 2 shows the schematic diagram of on-chip corpuscle separation. (201) is the fixture for centrifugal separation combined with the motor of (202), and, as for (203), the chip for corpuscle separation of W character-like passage, the corpuscle from which (204) was separated, and (205) show plasma. After corpuscle separation, the chip for corpuscle separation of (203) is countered, each chip for measurement of three markers of (206) is inserted in a location, and the hot platen (207) like the Peltier device further for temperature control is contacted at the rear face of each

chip for measurement of three markers who let the hole of (208) pass (206).
[0014]
Fig. 3 shows the schematic diagram of the measurement-of-gamma-glutamyl-transpeptidase approach. The chip for measurement of gamma-glutamyl transpeptidase of (301) is inserted in the jig (303) for on-chip centrifugal separation linking directly to the shaft (302) of a motor. (304) is a chip for W character-like corpuscle separation, (305) is an aponia needle, (306) shows the corpuscle after centrifugal separation and (307) shows plasma or a blood serum. (308) is a bypass for drawing prevention by blood plugging within a needle, and (309) is the cover and it removes it in the case of centrifugal separation. In this way, one side of two output port (310) and the intake (311) of the chip for measurement of gamma-glutamyl transpeptidase are combined in a pipe (312), and the plasma or the blood serum obtained at one side of W character-like passage is moved to the plasma of a mixer (313), or a blood serum reservoir (314) with the pipe (316) and external pump which were connected with inhalation opening of (315). This external pump may form and use an internal pump like an electroendosmose style pump. With this pump, the substrate buffer solution which consists of a L-gamma-glutamyl-p-N-ethyl-N-hydroxyethylamino anilide, a glycylglycine, and a 1-naphthol-2-sulfonic acid is introduced into a mixer (313) from eye a liquid pool (317) at coincidence. Then, plasma or a blood serum is introduced into a mixer (311) also from plasma or a blood serum reservoir (314). A mixed operation of the solution by the mixer concerned
[0018] It comes out and states.
[0015]
Periodic acid works as an oxidizer to the product to which the |-glutamyl transpeptidase enzyme acted on the substrate buffer solution, blue coloring matter is generated, and measurement of gamma-glutamyl transpeptidase is performed by the detection which is 660nm of the peak wavelength. Therefore, the detection system containing the light source and a photodetector has the concern which a measurement error produces by aging, fluctuation of substrate temperature, etc. The error reduction prepares two capillaries for spectrometry of the same configuration in one substrate, and solves them by pouring the reference liquid which mixed the substrate buffer solution and coloring liquid for the sample solution which mixed plasma, the substrate buffer solution, and coloring liquid in a sink and another side to one side, and measuring each absorbance. By making the capillary for spectrometry by the side of reference liquid into a reference at this time, the absorbance of a sample solution is obtained as an always amended value.
[0016]
Therefore, the one half of the mixed liquor obtained in this way is poured in with an external pump through length opening of (319) into the passage (318) for reference. Length opening (321) of the passage (320) for measurement is closed in that case. Next, it mixes by pouring periodic acid into a mixer (313) with an external pump from a coloring material reservoir of (322). This external pump may form and use an internal pump like an electroendosmose style pump. Then, length opening of (319) is closed, lets length opening of (321) pass, and pours the measurement liquid of the remaining half section into the passage (320) for measurement with an external pump. Spectrometry establishes the light source (323) of a high brightness red light emitting diode etc. on the passage near the exhaust port of (315), carries out incidence of the 660nm light through a band pass filter, and detects the light which has prepared and penetrated (324) of photodetectors, such as a silicon photodiode, on the passage near [each] length opening of (319) and (321).
[0017]
Fig. 4 shows the schematic diagram of the chip for GOT and GPT measurement. What is necessary is just to prepare it for each measurement one as passage, since measurement of GOT and GPT measures the reduction rate of the absorbance of the matter which plasma or a blood serum is added to the substrate buffer solution, and each enzyme acts, and is produced. The chip structure for |-glutamyl transpeptidases and the being similar difference which are fundamentally shown in Fig. 3 are only that reference and the passage for measurement have become one. the plasma or the blood serum for GOT and GPT measurement should also obtain

that of the passage for W character-like corpuscle separation of (401), and combine the output port of (402), and the intake (403) of the chip for measurement of GOT or GPT in a pipe (404) from one of the two, and pass a connection pipe (407) from inhalation opening (408) in a mixer (406) via the plasma of (405), or a corpuscle reservoir at it -- it is introduced from an external pump. The minute tungsten lamp with a band pass filter with which the passage for measurement and (410) pass the service entrance of measurement mixed liquor, and, as for (409), (411) passes light with a wavelength of 340nm, and (412) are silicon photodiodes.

[0018]

Fig. 5 shows the structure of a mixer, and the schematic diagram of a mixed approach. (501) is a mixer chip and is carried in the on-chip centrifugal separation machine of Fig. 2. (502) is the center of rotation. (503) is the inlet of plasma or a blood serum, and is accumulated in the plasma after impregnation (504), or the reservoir of a blood serum. (505) is the inlet of reagents, such as the substrate buffer solution, and is accumulated in the reagent reservoir of (506). First, although both liquid will move to the mixed container (a) of (508) according to a centrifugal force as shown in Fig. 5 (1) if it rotates for 5 seconds the rate for /5000 times to the hand of cut of (507), each other is in the condition of hardly being mixed. Next, as are shown in Fig. 5 (2), and it moves to the mixed container (b) leaned 90 degrees (509) and is shown in Fig. 5 (3) after rotating in the same direction for 5 seconds the rate for /5000 times, it moves from a mixed container (b) to a mixed container (a) again, and mixing of both liquid is completed after rotating in the same direction for 5 seconds the rate for /5000 times. In case a mixed container (a) and the reason for having prepared two or more areole in (b) mix a reagent with a blood serum, they are for increasing enlarging surface area which both liquid contacts, and the count of a collision contacting and mixing both liquid.

[0019]

Fig. 6 is a schematic diagram of the cross section of the passage (315) for reference, and the passage (317) for measurement shown in Fig. 3. In order to lengthen the optical path length the making absorption of incident light increase purpose, it is required to carry out total reflection of the light in passage in the object for reference, and the passage for measurement. Therefore, three items were invented. When the light from the light source (601) of light emitting diode, a tungsten lamp, etc. carries out incidence of one to passage (603) through a quartz aperture (602), the include angle of 45 degrees is prepared, the inside of passage is reflected and, as for the edge (604) of passage, another [in which the light (605) which passed carries out incidence to a quartz aperture (606) electric eye (607) from passage (603)] passage edge (608) also prepares the include angle of 45 degrees. (609) is the inlet of reference or measurement liquid, and (610) is an outlet. Furthermore, in order that light may carry out total reflection in passage, the wall of passage (602) is given a water-repellent finish. An approach applies to the front face of for example, a PET (polyethylene terephthalate) plate what distributed the Teflon particle to the isooctane. This manufacture process is mentioned later. It can overheat, after forming passage, and otherwise, in the case of the passage made from a quartz plate, it can overheat at about 250 degrees C, DMAH (dimethyl aluminum hydride) can be introduced in passage, and the coat of the wall can also be carried out with Aluminum CVD. Furthermore, the part of the passage for measurement may be made into the ground metal. Moreover, it was effective even if it used the opaque substrate to incident light.

[0020]

Fig. 7 shows the schematic diagram which looked at the passage (315) for reference, and the passage (317) for measurement from the top. In order to lengthen the optical path length, it is made the structure which folded up passage (701), and the bending section (702) prepares the include angle of 45 degrees, as shown in (703), and reflects light. If it does in this way, the passage of 100-micrometer width of face with a die length of 1cm can be formed in width of face of 1mm, and, as for at least five, can form the optical path length with an overall length of 5cm in it. This is one advantage of a microchip. Of course, this wall also performs the coat of Teflon membrane or a metal membrane (704).

[0021]

Figs. 8 are reference of the microchip concerned, and a schematic diagram of the manufacture

process of the part of the passage for measurement. (1) Form the reverse pattern of patterns, such as micro passage, with metal mold (801). (2) Carry out the mold of the metal mold pattern to polymer substrates (802), such as a PET (polyethylene terephthalate) plate, a polycarbonate plate, etc. of 2cm angle. For example, in the case of the PET plate, they were 95 degrees C, 0.15MPa, and the conditions for 3 minutes. (3) Next, carry out the coat of the fluororesin (803). (4) Grind the fluororesin which carried out the coat using 2-propanol to the flat side of PET, and leave a fluororesin only to the pars basilaris ossis occipitalis and the side-attachment-wall section of passage. (5) Carry out thermocompression bonding of the PET plate for a cap which prepared the inlet of the solution prepared similarly, the incidence (804) of the object for outlets (not shown), or light, and the hole of outgoing radiation opening (805) on 75 degrees C, 0.1MPa, and the conditions for 3 minutes. (6) Paste up a quartz aperture (806) on the incidence (804) of light, and the hole of outgoing radiation opening (805).

[0022]

[Example]

Fig. 9 (1), (2), and (3) show |-glutamyl transpeptidase and the each school forward curve of GOT and GPT. First, whole blood is extracted with an aponia needle, and it pours into W character passage with a pump, and stops for 30 minutes as it is, and by coagulation factors, such as FIBURINOGEN in blood, centrifugal separation on chip is performed after generating a clot, and it separates into a clot and a blood serum. At that time, the blood serum concerned was diluted with deionized water for this proofreading measurement, and it adjusted so that an activity value might serve as 0, 11, 22, 33, and 43 IU/l. Drawing 9 - In measurement of the |-glutamyl transpeptidase of (1), the L-gamma-glutamyl-p-N-ethyl-N-hydroxyethylamino anilide of 12 mmol/l concentration, the 1-naphthol-2-sulfonic acid of 0.2 mmol/l concentration, and 37 degrees C of substrate buffer solutions of 0.5ml2 mmol/l containing the glycylglycine of concentration 50 mmol/l are heated for 3 minutes by substrate buffer-solution reservoir, and it introduces into a mixer. And the blood serum into which the activity value was changed is picked out from a W character passage chip by the micropipette, the 0.01ml is introduced into a mixer, and the 37 degrees C of the mixed liquor concerned are heated for 15 minutes. This is first introduced into the passage for reference. Next, 8.8 mmol/l is further added for the periodic acid of coloring material to the above-mentioned substrate buffer solution generated similarly and the mixed liquor of a blood serum, and it mixes by the mixer. This is introduced into the passage for measurement. The result in the passage for reference is changeless to the activity value of each |-glutamyl transpeptidase, it was shown that the detection system of the high brightness red LED and a photodiode is stable, and it was shown that it can measure in the near range of adult's normal values from a measurement result. Moreover, Fig. 9 (2) and (3) showed the calibration curve of GOT and GPT, and they performed it in this measurement with the UV method which used the beta-nicotinamide adeninedinucleotide reduction type. Adjustment and measurement temperature of mixed liquor were kept at 35 degrees C, made light emitted from the minute tungsten filament lamp the peak wavelength of 340nm with the band pass filter, irradiated the passage for measurement, detected the attenuance for 2 minutes with the silicon photodiode, and measured each concentration. Consequently, it was shown that it can measure in the near range of adult's normal values similarly.

[0023]

[Effect of the Invention]

After leading the blood of ultralow volume on hemanalysis equipment with the aponia needle this invention and performing separation of a corpuscle component, a blood serum, or a plasma component on it, the blood serum concerned or the plasma component was mixed with various reagents, and it was shown that the |-glutamyl transpeptidase which is an index showing a liver function, and GOT and GPT can be measured with a colorimetric method. What required long inspection time amount using large-sized and expensive conventional equipment by this is considered that everybody can inspect easily at home in a short time, and can contribute to prophylaxis as a result.

[Brief Description of the Drawings]

[Fig. 1] The schematic diagram of the equipment which extracts blood from an aponia needle and

is poured into the chip for corpuscle separation.
[Fig. 2] The schematic diagram of an on-chip corpuscle separation method.
[Fig. 3] The schematic diagram of the measurement-of-gamma-glutamyl-transpeptidase approach.
[Fig. 4] The schematic diagram of the chip for GOT and GPT measurement.
[Fig. 5] The structure of a mixer, and the schematic diagram of a mixed approach.
[Fig. 6] The schematic diagram of the cross section of the passage for reference, and the passage for measurement.
[Fig. 7] The schematic diagram which looked at the passage for reference, and the passage for measurement from the top.
[Fig. 8] Reference of the microchip concerned, and the schematic diagram of the manufacture process of the part of the passage for measurement.
[Fig. 9] (1) The each school forward curve of |-glutamyl transpeptidase, (2) GOT, and (3) GPT is shown.
[Description of Notations]
(101) Aponia needle
(102) Cylinder tubing
(103) Corpuscle separation chip
(104) Suction jig
(105) The pipe connected with an external pump
(106) W character-like passage
(107) Electrode holder
(108) The jig which performs insertion of a corpuscle separation chip and ejection
(109) Bypass
(201) The fixture for centrifugal separation combined with the motor
(202) Motor
(203) The chip for corpuscle separation of W character-like passage
(204) The separated corpuscle
(205) Plasma
(206) Each chip for measurement of three markers
(207) Peltier device
(208) Aperture
(301) The chip for measurement of gamma-glutamyl transpeptidase
(302) The shaft of a motor
(303) The jig for on-chip centrifugal separation
(304) The chip for W character-like corpuscle separation
(305) Aponia needle
(306) The corpuscle after centrifugal separation
(307) Plasma or a blood serum
(308) The bypass for drawing prevention by blood plugging within a needle
(309) Cover
(310) Two output port
(311) Intake of the chip for measurement of gamma-glutamyl transpeptidase
(312) Pipe
(313) Mixer
(314) Plasma or a blood serum reservoir
(315) Inhalation opening
(316) Pipe
(317) Eye a liquid pool
(318) Passage for reference
(319) Length opening
(320) Passage for measurement
(321) Length opening
(322) Color coupler reservoir

(323) The light source of high brightness red light emitting diode etc.
(324) Photodetectors, such as a silicon photodiode
(401) Passage for W character-like corpuscle separation
(402) Output port
(403) Intake of the chip for measurement of GOT or GPT
(404) Pipe
(405) Plasma or a corpuscle reservoir
(406) Mixer
(407) Connection pipe
(408) Inhalation opening
(409) Passage for measurement
(410) The service entrance of measurement mixed liquor
(411) Minute-with band pass filter tungsten lamp
(412) Silicon photodiode
(501) Mixer chip
(502) Center of rotation
(503) The inlet of plasma or a blood serum
(504) Plasma or the reservoir of a blood serum
(505) The inlet of a reagent
(506) Reagent reservoir
(507) Hand of cut
(508) A mixed container (a)
(509) A mixed container (b)
(601) Light source
(602) Quartz aperture
(603) Passage
(604) The edge of passage
(605) Light which passed
(606) Quartz aperture
(607) Electric eye
(608) Passage edge
(609) Inlet
(610) Outlet
(701) Passage
(702) Bending section
(703) 45-degree bending section
(704) Teflon membrane and a metal membrane
(801) Metal mold
(802) Polymer substrate
(803) Fluororesin
(804) Incidence opening of light
(805) Outgoing radiation opening of light
(806) Quartz aperture

[Translation done.]

* NOTICES *

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Fig. 1] The schematic diagram of the equipment which extracts blood from an aponia needle and is poured into the chip for corpuscle separation.
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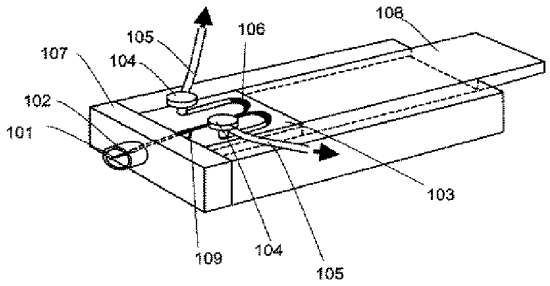
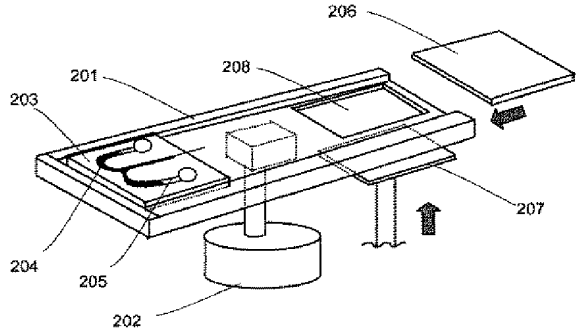
DRAWINGS

[Drawing 1]



[Drawing 2]



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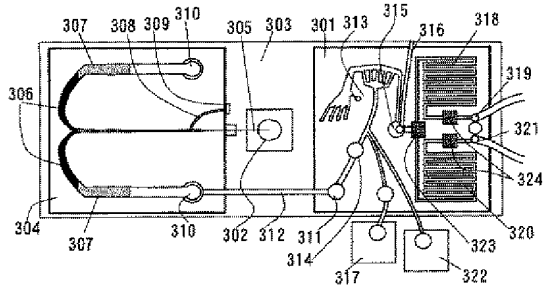

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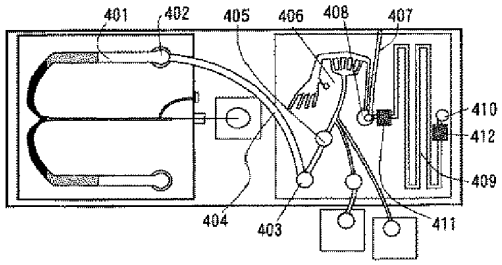

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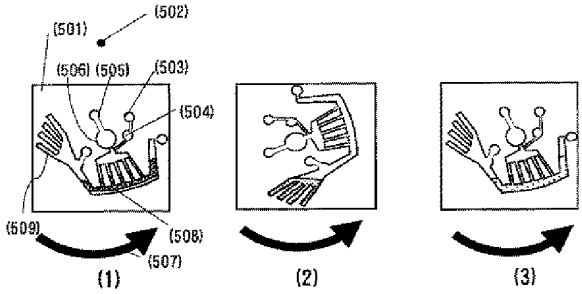

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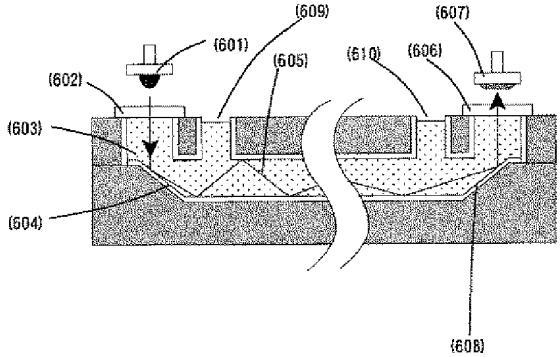

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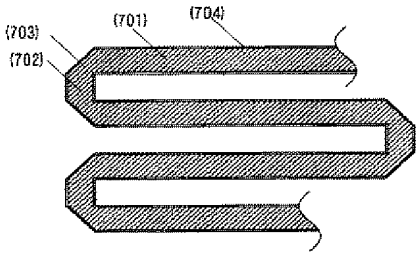

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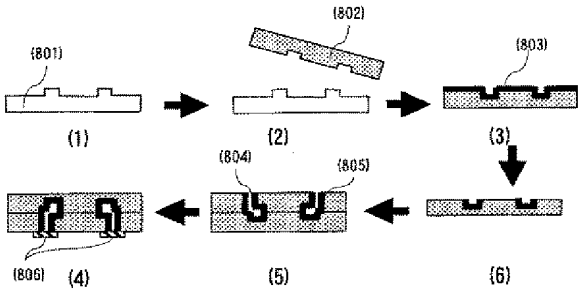
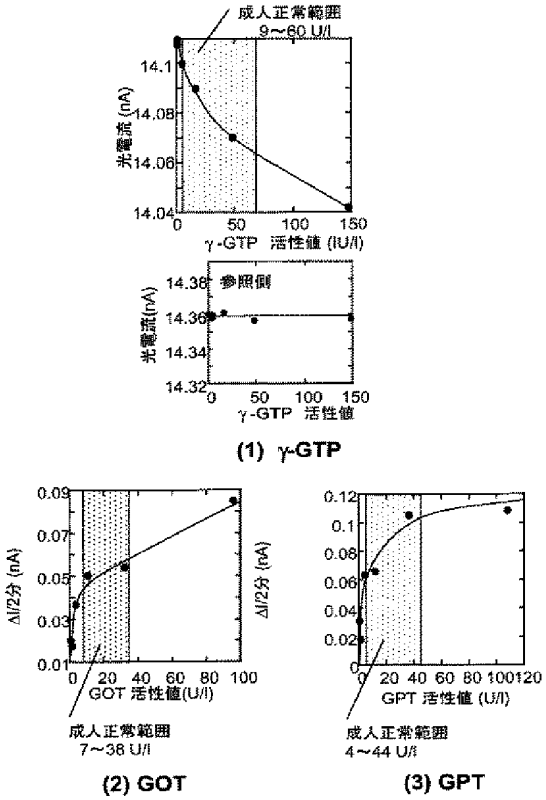


[Drawing 9]



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G01N 33/52	G01N 33/50	Z	

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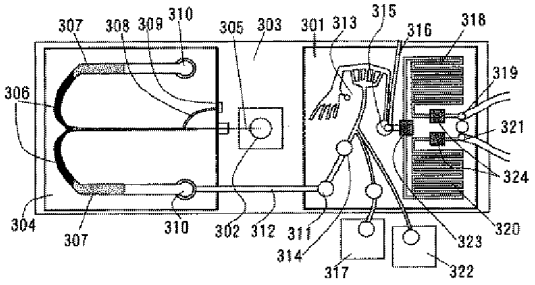
最終頁に続く

(54)【発明の名称】血液分析方法、血液分析装置および血液分析装置の製造方法

(57)【要約】
【課題】従来のヒトの肝機能を調べる指標であるγ－ＧＴＰ、ＧＯＴ、ＧＰＴを調べるためには多量の血液の採取と大型かつ高価な装置を用い、且つ時間のかかるものであった。
【解決手段】チップ上に採血のための無痛針、血球、血漿あるいは血清分離のためのＵ字キャピラリ、血漿あるいは血清および試薬の微細流路、遠心力を利用した混合機、光の光路を配置し、少量の血液からγ－ＧＴＰ、ＧＯＴ、ＧＰＴの濃度を短時間に測定することを可能とした。
装置の小型化、廉価化を図るために細胞導入口、流路、分岐流路およびポンプ手段を一体のチップ上に形成し、かつ特にポンプ手段には細胞に直接電界を印加することの無い電気浸透流ポンプを用いる。
【選択図】図３



【特許請求の範囲】
【請求項１】
一つあるいは複数の基板内に、生体内より血液を採取する手段と、少なくとも採取した当該血液をろ過し血漿または血清を得るろ過手段あるいは当該血液から血漿または血清を分離する手段の内いずれかの手段と、当該血漿または血清と基質緩衝液と発色剤を少なくともその２項目を混合する手段と、血漿または血清を当該混合手段にポンプにより移動させる手段と、当該混合液を測定用流路に移動させる手段と、測定用流路の一端には光照射装置、他の一端には光検出器を備える照射光の透過度を測定手段を備えたことを特徴とする血液分析装置。
【請求項２】
請求項１記載の血液分析装置は、肝機能の診断のマーカとなるγ－ＧＴＰとＧＯＴとＧＰＴの３酵素の濃度を測定することを特徴とする血液分析装置。
【請求項３】
請求項１記載の血漿または血清を分離する手段は、特にW字型の流路で構成されていることを特徴とする血液分析装置。
【請求項４】
請求項３記載のW型流路において、血液がはじめに導入される真中の流路の断面積が、両側の流路のそれよりも小さいことを特徴とする血液分析装置。
【請求項５】
請求項１記載の血液を採取する手段と、ろ過手段あるいは分離する手段との間の流路が分岐され血液分析装置外へと通ずるバイパス流路が構成されていることを特徴とする血液分析装置。
【請求項６】
請求項１記載の血液分析装置を構成する基板がポリマー樹脂で構成されていることを特徴とする血液分析装置。
【請求項７】
請求項６記載のポリマー樹脂が特に光学的に不透明なものであることを特徴とする血液分析装置。
【請求項８】
請求項１記載の混合する手段は、被混合液体を遠心力により血液分析装置内を移動、撹拌させることで均質に混合することを特徴とした混合方法。
【請求項９】
請求項１記載の血液を移動させる手段は、特に血液分析装置内に構成した電気浸透流ポンプであること特徴とする血液分析装置。
【請求項１０】
請求項１記載の血液を移動させる手段は、特に血液分析装置外のポンプであること特徴とする血液分析装置。
【請求項１１】
請求項１および２に記載のγ－ＧＴＯ測定用流路は、特に複数の同一形状の吸光度測定用流路から構成されることを特徴とする血液分析装置。
【請求項１２】
請求項１記載の測定用流路の表面の少なくとも一部は、テフロンを主成分とする材料で覆われていることを特徴とする血液分析装置。
【請求項１３】
請求項１記載の測定用流路の表面の少なくとも一部は、金属を主成分とする材料で覆われていることを特徴とする血液分析装置。
【請求項１４】
請求項１記載の測定用流路を構成する側壁の少なくとも一箇所には、測定光の進行方向に対して法線ベクトルが約４５度となる面を含むことを特徴とする血液分析装置。
【請求項１５】

請求項１記載の血液分析装置は、遠心分離装置上で当該血液分析装置の温度を制御することを特徴とする血液分析方法。
【請求項１６】
請求項１５記載の血液分析装置の温度を制御はペルチェ素子を当該血液分析装置に接触させて行うことを特徴とする血液分析方法。
【請求項１７】
請求項１記載の血液分析装置は、遠心分離装置上で血液から血漿または血清を分離して得、引き続き当該遠心分離装置上で当該血漿または血清を種々の試薬と混合した後に測定用流路において血液中の成分分析を行うことを特徴とする血液分析方法。
【請求項１８】
請求項１記載の血液分析装置の血液を採取する手段を被採取部位の皮膚と接触させるときに、当該皮膚採血部位と血液を採取する手段の少なくとも一部の周囲を減圧雰囲気とすることを特徴とする血液分析方法。
【請求項１９】
血液分析装置を設置し、請求項１８記載の減圧雰囲気を維持するための血液を採取する手段の少なくとも一部を外界と隔離する槽と当該槽内を減圧とするためのポンプとの接続手段を有し、かつ血液を採取する手段の少なくとも一部が皮膚内へと進入する方向へと血液分析装置を移動させることのできる機構を有する血液採取装置。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】
本発明は、痛みを伴わない無痛針より血液を採取し、遠心分離を行って得た血漿または血清に基質緩衝液混ぜ、更に発色液を添加した混合液から生じる色素の吸光度を測定する、あるいは混ぜた基質緩衝液内で生じる反応生成物濃度の減少速度を吸光度で測定する、つまり比色法によって肝機能を測定する装置ならびに製作方法に関する。特に、これらの少なくとも一つの機能が少なくとも一体のチップ基板上に集積されており、さらにそのチップ基板は小さく、その取り扱いに専門の医学の知識、資格が必要とせず、簡単に上述の肝機能診断を短時間に行うことを特徴とする肝機能診断デバイスに関する。
【０００２】
【従来の技術】
人間のの肝機能は、ＧＰＴ（Ｇｌｕｔａｍｉｃ－ｐｙｒｕｖｉｃ ｔｒａｎｓａｍｉｎａｓｅ）、ＧＯＴ（Ｇｌｕｔａｍｉｃ－ｏｘａｌｏａｃｅｔｉｃ ｔｒａｎｓａｍｉｎａｓｅ）、γ－ＧＴＰ（γ－Ｇｌｕｔａｍｙｌｔｒａｎｓｐｅｐｔｉｄａｓｅ）の３つの酵素の値を測定することで診断されている。これら３つの酵素は生体内において特に肝臓に多く存在しており、通常血清中の酵素活性値は一定に保たれている。例えば、ＧＯＴは８～４８ＩＵ／リットル、ＧＰＴは１３～３５ＩＵ／リットル、γ－ＧＴＰ：７～６０ＩＵ／リットルの範囲が成人の正常値範囲である。ここでＩＵはＩｎｔｅｒｎａｔｉｏｎａｌ Ｕｎｉｔを示す。しかし肝障害等を引き起こすと血清中のこれらの活性値が上昇することが知られている。従って、３つのマーカを測定することは肝機能診断、並びに治療方針の決定、治療経過の観察に不可欠である。
【０００３】
これらの３つのマーカの検査は、他の多くの分析項目と共に自動血液分析検査で行われる。汎用の自動血液分析検査は、注射器により採血したミリリットルの多量の血液を用い、試料分取計量系、試薬分取計量系、反応系、比色法を基本とした検知測定系、洗浄系、制御・データ処理系からなる大規模かつ高価な装置である。しかも、肝機能のみの検査でも本装置を使わざるを得ないのが現状であり、しかも検査結果が出るまで長時間を要する。
【０００４】
一方、半導体製品に伴う微細加工技術の進歩を土台に、近年、μ－ＴＡＳ（ＭｉｃｒｏＴｏｔａｌ Ａｎａｌｙｔｉｃａｌ Ｓｙｓｔｅｍ）や、Ｌａｂ．ｏｎａ Ｃｈｉｐ（チップ上の研究施設）と言われるような、数ｃｍ四方の平板の基板上に種々の薬液の微小流路

や微小分析器を配置し、その基板上で種々の化学合成や物理化学分析を行う試みが盛んに行われている。このような、指の先と同寸法のチップ上で前記の化学合成、分析を行う利点として、従来のいわゆる化学実験室のように広い空間や大規模・高価な測定装置を必要とせず、チップ自体が可搬であること、使用する薬液量や分析に必要とされる電力量が著しく少なくなり、環境に優しいなどが挙げられる。このようなチップの一つの大きな応用として、無痛針より採取した極微量の血液をチップ上の数十～数百μｍの幅と深さを有するマイクロ流路に導入し、当該チップ上において血液から血球を分離し、得られた血漿から健康マーカを測定するヘルスケアチップ（特願２００１－２５８８６８）や、ＤＮＡを高速で分離するチップ、プロテインチップなどが、極細の流路内で流体が動くことに基づく微小流体力学（ｍｉｃｒｏｆｌｕｉｄｉｃｓ）の基礎として盛んに研究・開発されている。

【０００５】

このμ－ＴＡＳの一応用として、一つの小寸法の基板に、無痛針より採取した極微量の血液をチップ上のマイクロ流路に導入し、その血漿または血清から肝機能診断が可能なマイクロチップが実現すると、患者をベッドサイドで迅速に検査でき、医者により患者の容態が短時間に把握でき、適切な診察をもたらすのみならず、在宅において、アルコール摂取量や食事の種類、スポーツの必要などに対する健康管理を可能にし、更には病状の診断にも大いに役立つと期待される。また、比色法を用いた本発明は、グルコースやクレアチニンや尿素窒素などの生活習慣病のマーカの測定にも応用でき、在宅での健康管理用のヘルスケアチップやベッドサイドでの緊急の多項目を診断する検出器にも展開できる。

【０００６】

【発明が解決しようとする課題】

マイクロチップを用いて肝機能を簡便・迅速に測定するためには、まず使い捨て可能な安価なポリマーのような基板を用いてチップを作製しなければならない。また、ＧＰＴ、ＧＯＴ、γ－ＧＴＰの３マーカを高感度・高精度に測定するためには、γ－ＧＴＰ測定では、血漿または血清に基質緩衝液と発色液を混合しなければならない。ＧＰＴとＧＯＴ測定でも血漿または血清中に基質緩衝液を混ぜ、生じた反応生成物の濃度の減少速度を吸光度で測定する。従って、当該計測法では溶液の混合が不可欠である。しかし、μｍ寸法のマイクロ流路中での溶液は、対流より粘性が大きくなり、即ちレイノルズ数が低くなるため、マイクロ流路中での溶液の流れは層流が支配的になる。それ故に、複数の液をマイクロ流路の中に導入しても、各々が混ざらずに独自に流れ、混合が非常に困難である。また、３マーカを比色法で測定する際、γ－ＧＴＰ測定では、発色液を添加した混合液から生じる色素の濃度を測るため、光源と光検出器を含む検出系の経時変化、基板温度のゆらぎなどにより測定誤差が生じる。ＧＰＴとＧＯＴ測定では、反応生成物の濃度を、マイクロ流路内でにそれぞれの化学結合のエネルギに対応した波長の光の吸光度測定が求められる。これらの吸光度を高Ｓ／Ｎ比で測定するためには、光がマイクロ流路内でできるだけ長い距離を通過し、当該色素や反応生成物により吸収させねばならない。そのためには、照射光がマイクロ流路の内壁で全反射することが要求される。

【０００７】

本発明の目的は、使い捨て可能な安価な基板を用いてチップの作製方法と、マイクロチップ内で血漿または血清に基質緩衝液や発色液を混合し、検出系の経時変化などによる測定誤差を補正する方法と、照射光がマイクロ流路の外部に漏れることなく全て流路内で閉じ込める方法を提供することにある。

【０００８】

【課題を解決させるための手段】

痛みを伴わない無痛針より血液を採取して、W字状の流路に注入し、その流路内で遠心分離を行って得た血漿または血清に、γ－ＧＴＰの場合は、基質緩衝液と発色材を混ぜ、遠心力を用いて混合し、当該混合液から生じる色素の吸光度を測定し、あるいＧＴＰやＧＯＴの場合は、遠心力を用いて混合した基質緩衝液内で生じる反応生成物濃度の減少速度を吸光度で測定し、その際、入射光の色素による吸収を促進するため、入射光が効率よく流

路に入り、出射させるように流路の両端は４５度の角度を持たせ、更に光路長を長くするため流路の折れ曲がれ領域では、流路の側壁は４５度の角度にし、流路の内壁にテフロン膜などをコートして全反射をさせることにより高精度・高感度に肝機能を測定する使い捨て可能な安価な基板を用いてチップを作製する。

【発明の実施の形態】

【０００９】

まず初めに、γ－ＧＴＰとＧＯＴとＧＰＴの測定原理を説明する。これらは濃度ｃの溶液に強度Ｉ$_0$の光が入射され、長さＬを透過後、光の強度がＩに減衰したとき、透過度はｔはＩ／Ｉ$_0$＝１０$^{-k_0 L}$になり、光路長Ｌが一定のとき、吸光度Ｅは－１ｎ（Ｉ／Ｉ$_0$）＝ｋ$_0$ｃ（ｃは常数）となる。これをＢｅｅｒの法則という。γ－ＧＴＰの測定の場合は、γ－ＧＴＰ酵素の作用により生成される色素を定量することにより、γ－ＧＴＰの活性量を求める。実際の測定では、例えばＨＡ基質法と呼ばれるＬ－γ－グルタミル－ｐ－Ｎ－エチル－Ｎ－ヒドロキシエチルアミノアニリドとグリシルグリシンの基質緩衝液に血漿または血清を加えた混合液に血漿中のγ－ＧＴＰ酵素が作用するとｐ－Ｎ－エチル－Ｎ－ヒドロキシエチルアミノアニリンが生成する。これと基質緩衝液中の１－ナフトール－２－スルホン酸が反応し、更に過ヨウ素酸が酸化剤として働き、青色色素を発生する。これは６６０ｎｍにピークを有する吸収スペクトルを示し、６６０ｎｍの光を照射して、その吸光度Ｅを測定する（参考文献；藤沢　洌、他：日本臨床（春季創刊号）３８，８８９－８９５））。

【００１０】

ＧＯＴの測定原理は、Ｌ－アスパラギン酸とα－ケトグルタル酸を含む基質緩衝液に血漿または血清を加えると、血漿中のＧＯＴ酵素が作用してグルタミン酸とオキザロ酢酸を生成する。生成したオキザロ酢酸はβ－ニコチンアミドアデニンジヌクレオチド還元型（ＮＡＤＨ）の存在下でリンゴ酸脱水素酵素（ＭＤＨ）の作用によってリンゴ酸に変化する。このときＮＡＤＨはβ－ニコチンアミドアデニンジヌクレオチド酸化型（ＮＡＤ）に酸化され３４０ｎｍの吸光度が減少する。この減少速度を測定することにより血漿中のＧＯＴ活性値を求める。ＧＰＴの測定原理は、Ｌ－アラニンとα－ケトグルタル酸を含む基質緩衝液に血漿を加えると試料中のＧＰＴ酵素が作用してグルタミン酸とピルビン酸を生成する。生成したピルビン酸はβ－ニコチンアミドアデニンジヌクレオチド還元型（ＮＡＤＨ）の存在下で乳酸脱水素酵素（ＬＤＨ）の作用によって乳酸に変化する。このときＮＡＤＨはβ－ニコチンアミドアデニンジヌクレオチド酸化型（ＮＡＤ）に酸化され３４０ｎｍの吸光度が減少する。この減少速度を測定することにより血漿中のＧＯＴ活性値を求める（参考文献；　斉藤正行、丹波正治（編）、「臨床化学」、講談社（１９８１）１１６）。

【００１１】

上述の測定原理を基に、γ－ＧＴＰ、ＧＰＴ、ＧＯＴの３マーカをマイクロチップにより測定する本発明をより詳細に説述するため、添付の図面に沿ってこれを説明する。

【００１２】

第１図は、人の皮膚に無痛針を刺して血液を採取し、血球分離用チップに注入する装置の概略図を示す。（１０１）は無痛針であり、約９０μｍ径のステインレス管の先端を１０度で研磨しも更に電解研磨で鋭利にしたもので、細いため痛みをほとんど感じない。（１０２）は、円筒管であり、最初に皮膚に接触し、その接触感から痛みがあっても和らげる役目を果たすと共に、（１０３）の血球分離チップに設けられた（１０４）の吸引冶具から外部ポンプにより血液をチップに引き込み器と連動して、円筒管に接触した皮膚表面を減圧にして皮膚を盛り上がらせ、針が自動的に皮膚に刺さる。この外部ポンプの代わりに、電気浸透流ポンプのような小型かつ高吐出なポンプ血液分析装置と一体に設けて使用してもよい。（１０５）は外部ポンプと接続されるパイプである。（１０６）はチップのＷ字状流路を、（１０７）はホルダー、（１０８）は血球分離チップの挿入と取り出しを行う冶具を示す。（１０９）はバイパスを示す。これを設けた理由は、後述のチップ上で血球の分離を遠心力で行う際（オンチップ血球分離と呼ぶ）、無痛針内に溜まっている血液

が遠心分離中に凝固して針内で詰まってしまい、得られた血漿をチップ内から外部にポンプで引き出せなくなるからである。採血中にはこのバイパスの端はスポンジのようなものを押し付けつことで血液分析装置外部の雰囲気とは遮断され、機密性を得ている。また、（１０６）の血球分離用のチップの流路がW字状になっている理由を以下に述べる。即ち、Ｕ字状流路では遠心分離後に血球はＵ字状流路の底部に溜まり、血漿は両側の流路で得られるが、片側だけでは得られる血漿量が制限される。一方、W字状はＵ字状の一流路を共有し、それを細くして無痛針から全血を導入し、両側の太い流路に全血が満たされた後、遠心分離を行うと、両側の流路により多くの血漿が得られる。また、この血球分離チップはＰＥＴ（ポリエチレンテレフタレート）のようなポリマー基板で製作されるが、ＰＥＴ製の流路の中に全血が導入されると直ちに赤血球やたんぱく質が流路の内壁に付着し、更には凝固も起こる。それを防ぐため、ＭＰＣ（２－ｍｅｔｈａｃｒｙｌｏｙｌｏｘｙｅｔｈｙｌｐｈｏｓｐｈｏｒｙｌｃｈｏｌｉｎｅ；参考文献Ｋ．Ｉｓｈｉｈａｒａ，Ｈ．Ｏｓｈｉｄａ，Ｔ．Ｕｅｄａ，Ｙ．Ｅｎｄｏ，Ａ．Ｗａｔａｎａｂｅ ａｎｄ Ｎ．Ｎａｋａｂａｙａｓｈｉ：Ｊ．Ｂｉｏｍａｔ．Ｍａｔ．Ｒｅｓ：２６（１９９２）１５４３．）ポリマーを内壁にコートする。なお、血球分離はオンチップ遠心分離を行った記述をしたが、勿論特願２００１－２５８８６８に示した寸法の異なる微細ピラーを流路内に設けるろ過手段を用いてことができる。

【００１３】

第２図は、オンチップ血球分離の概略図を示す。（２０１）は、（２０２）のモータと結合した遠心分離用治具であり、（２０３）はW字状流路の血球分離用チップ、（２０４）は分離された血球、（２０５）は血漿を示す。血球分離後、（２０６）の３マーカの各測定用チップを（２０３）の血球分離用チップに対向して位置に挿入し、更に温度制御用のペルチェ素子の如き熱板（２０７）を（２０８）の孔を通して（２０６）の３マーカの各測定用チップの裏面に接触させる。

【００１４】

第３図は、γ－ＧＴＰ測定方法の概略図を示す。（３０１）のγ－ＧＴＰ測定用チップをモータの軸（３０２）に直結したオンチップ遠心分離用治具（３０３）に挿入する。（３０４）はW字状血球分離用チップであり、（３０５）は無痛針であり、（３０６）は遠心分離後の血球、（３０７）は血漿または血清を示す。（３０８）は針内での血液詰まりによる引き抜き防止用のバイパスであり、（３０９）はそのふたであり、遠心分離の際には取り外す。こうしてW字状流路の片側で得られた血漿または血清を、２箇所の取り出し口（３１０）の一方とγ－ＧＴＰ測定用チップの取り入れ口（３１１）をパイプ（３１２）で結合し、ミキサー（３１３）の血漿または血清溜め（３１４）に、（３１５）の吸入口に繋がったパイプ（３１６）と外部ポンプによって移動させる。この外部ポンプは電気浸透流ポンプのような内部ポンプを設けて使用してもよい。同時にこのポンプによって、液溜め（３１７）からＬ－γ－グルタミル－ｐ－Ｎ－エチル－Ｎ－ヒドロキシエチルアミノアニリドとグリシルグリシンと１－ナフトール－２－スルホン酸からなる基質緩衝液をミキサー（３１３）に導入する。その後、血漿または血清溜め（３１４）からも血漿または血清をミキサー（３１１）に導入する。当該ミキサーによる溶液の混合作用は【００１８】で述べる。

【００１５】

γ－ＧＴＰ測定は、基質緩衝液にγ－ＧＴＰ酵素が作用した生成物に過ヨウ素酸が酸化剤として働き、青色色素が発生し、そのピーク波長の６６０ｎｍの検出により行われる。従って、光源と光検出器を含む検出系に経時変化や基板温度のゆらぎなどにより測定誤差が生じる懸念がある。その誤差低減は、一つの基板内に同一形状の吸光度測定用キャピラリを２本設け、一方には血漿と基質緩衝液と発色液を混合した試料液を流し、他方には基質緩衝液と発色液を混合した参照液を流してそれぞれの吸光度を測定することにより解決する。このとき参照液側の吸光度測定用キャピラリをリファレンスとすることで、試料液の吸光度は常に補正された値として得られる。

【００１６】

従って、こうして得られた混合液の半分を参照用流路（３１８）の中に（３１９）の引き口を通して外部ポンプで注入する。その際、測定用流路（３２０）の引き口（３２１）は閉じておく。次に、（３２２）の発色材溜めから過ヨウ素酸をミキサー（３１３）に外部ポンプにより注入し、混合を行う。この外部ポンプは電気浸透流ポンプのような内部ポンプを設けて使用してもよい。その後、（３１９）の引き口は閉じ、（３２１）の引き口を通して、残り半部の測定液を測定用流路（３２０）に外部ポンプで注入する。吸光度測定は、（３１５）の排気口付近の流路上に高輝度赤色発光ダイオードなどの光源（３２３）を設け、バンドパスフイルターを通して６６０ｎｍの光を入射し、（３１９）と（３２１）の各引き口付近の流路上にシリコンフォトダイオードなどの光検出器の（３２４）を設け、透過してきた光を検出する。

【００１７】

第４図は、ＧＯＴとＧＰＴ測定用のチップの概略図を示す。ＧＯＴとＧＰＴの測定は、基質緩衝液に血漿または血清を加えて各酵素が作用して生じる物質の吸光度の減少速度を測定するので、流路としては各測定に一本用意すればよい。基本的には第３図に示すγ－ＧＴＰ用のチップ構造と類似し、違いは参照と測定用流路が一本になっているのみである。ＧＯＴとＧＰＴ測定のための血漿または血清は、（４０１）のＷ字状血球分離用流路のもう片方から、（４０２）の取り出し口とＧＯＴまたはＧＰＴの測定用チップの取り入れ口（４０３）をパイプ（４０４）で結合し、（４０５）の血漿または血球溜めを経由してミキサー（４０６）に吸入口（４０８）からそれに接続パイプ（４０７）を経て外部ポンプより導入される。（４０９）は測定用流路、（４１０）は測定混合液の引き込み口、（４１１）は３４０ｎｍの波長の光を通過させるバンドパスフィルター付の微小タングステンランプ、（４１２）はシリコンフォトダイオードである。

【００１８】

第５図は、ミキサーの構造と混合方法の概略図を示す。（５０１）はミキサーチップであり、第２図のオンチップ遠心分離器に搭載する。（５０２）はその回転中心である。（５０３）は血漿または血清の注入口であり、注入後（５０４）の血漿または血清のリザーバに溜める。（５０５）は基質緩衝液などの試薬の注入口であり、（５０６）の試薬リザーバに溜める。まず、（５０７）の回転方向に５０００回／分の速度で５秒間回転すると、第５図（１）に示すように、遠心力により（５０８）の混合容器（ａ）に両液が移動するが、お互いがほとんど混ざらない状態になっている。次に、第５図（２）に示すように、９０度傾けて（５０９）の混合容器（ｂ）に移して、５０００回／分の速度で５秒間同じ方向に回転後、第５図（３）に示すように、混合容器（ｂ）から再び混合容器（ａ）に移し、５０００回／分の速度で５秒間同じ方向に回転後、両液の混合が完了する。混合容器（ａ）と（ｂ）に複数の小室を設けた理由は、血清と試薬を混合する際、両液が接触する表面積を大きくすることと、両液が接触して混ざるための衝突回数を増すためである。

【００１９】

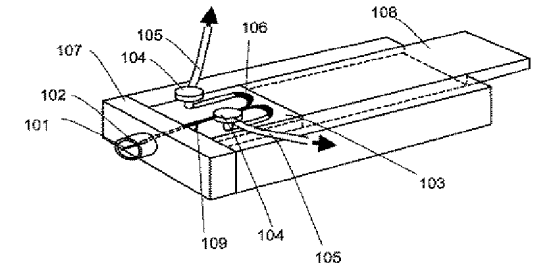
第６図は、第３図で示した参照用流路（３１５）と測定用流路（３１７）の断面の概略図である。参照用と測定用流路では、入射光の吸収を増加させる目的で光路長を長くするため、光を流路内で全反射させることが必要である。そのために３項目を発明した。一つは、発光ダイオードやタングステンランプなどの光源（６０１）からの光が石英窓（６０２）を通して流路（６０３）に入射するとき、流路の端（６０４）は４５度の角度を設け、流路内を反射し、通過した光（６０５）が流路（６０３）から石英窓（６０６）受光器（６０７）に入射するもう一方の流路端（６０８）も４５度の角度を設ける。（６０９）は参照または測定液の注入口、（６１０）は出口である。更に、流路内で光が全反射するために、流路（６０２）の内壁を撥水処理する。方法は、例えばＰＥＴ（ポリエチレンテレフタレート）板の表面に、テフロン微粒子をイソオクタンに分散したものを塗布する。この製造プロセスは後述する。他に、石英板製の流路の場合は、流路を形成後、２５０℃程度に過熱し、ＤＭＡＨ（ジメチルアルミハイドライド）を流路内に導入して内壁をアルミＣＶＤでコートすることもできる。更には、測定用流路の部分は研磨した金属製にしてもよい。また、入射光に対して不透明な基板を用いても効果があった。

【００２０】
第７図は、参照用流路（３１５）と測定用流路（３１７）を上から見た概略図を示す。光路長を長くするためには、流路（７０１）を折りたたんだ構造にし、曲げ部（７０２）は（７０３）のように４５度の角度を設けて光を反射させる。このようにすると、１ｃｍの長さの１００μｍ幅の流路は１ｍｍの幅に少なくとも５本は形成でき、全長５ｃｍの光路長が形成できる。これはマイクロチップの一利点である。勿論この内壁もテフロン膜や金属膜（７０４）のコートを行う。
【００２１】
第８図は、当該マイクロチップの参照と測定用流路の部分の製造プロセスの概略図である。（１）マイクロ流路などのパターンの逆パターンを金型（８０１）で形成する。（２）その金型パターンを２ｃｍ角のＰＥＴ（ポリエチレンテレフタレート）板やポリカーボネート板などのポリマー基板（８０２）にモールドする。例えばＰＥＴ板の場合、９５℃、０．１５ＭＰａ、３分の条件であった。（３）次に、フッ素樹脂（８０３）をコートする。（４）ＰＥＴの平坦面にコートしたフッ素樹脂を２－プロパノールを用いて研磨し、流路の底部と側壁部にのみフッ素樹脂を残す。（５）同様に用意した溶液の注入口、出口用（図示せず）や光の入射（８０４）、出射口（８０５）の孔を設けたキャップ用ＰＥＴ板を、７５℃、０．１ＭＰａ、３分の条件で熱圧着する。（６）石英窓（８０６）を光の入射（８０４）、出射口（８０５）の孔の上に接着する。
【００２２】
【実施例】
第９図（１）、（２）、（３）は、γ－ＧＴＰ、ＧＯＴ、ＧＰＴの各校正曲線を示す。まず、全血を無痛針で採取してポンプによってＷ字流路に注入し、そのまま３０分間留め、血液内のフィブリノゲーンなどの凝固因子によって血餅を生成後、オンチップ遠心分離を行い、血餅と血清に分離する。その際、本校正測定のため当該血清を脱イオン水で希釈し、活性値が０，１１，２２，３３，４３　ＩＵ／ｌとなるよう調整した。図９－（１）のγ－ＧＴＰの測定では、基質緩衝液溜めで１２ｍｍｏｌ／ｌ濃度のＬ－γ－グルタミル－ｐ－Ｎ－エチル－Ｎ－ヒドロキシエチルアミノアニリドと０．２ｍｍｏｌ／ｌ濃度の１－ナフトール－２－スルホン酸と濃度５０ｍｍｏｌ／ｌのグリシルグリシンを含んだ０．５ｍｌ２ｍｍｏｌ／ｌの基質緩衝液を３７℃，３分間加熱して、ミキサーに導入する。そして活性値を変えた血清をマイクロピペットでＷ字流路チップから取り出し、その０．０１ｍｌをミキサーに導入し、当該混合液を３７℃、１５分間加熱する。まずこれを参照用流路に導入する。次に同様に生成した前述の基質緩衝液と血清の混合液に更に発色材の過ヨウ素酸を８．８ｍｍｏｌ／ｌを加えてミキサーによって混合する。これを測定用流路に導入する。参照用流路での結果は各γ－ＧＴＰの活性値に対し変化が無く、高輝度赤色ＬＥＤとフォトダイオードの検出系が安定であることを示し、測定結果から成人の正常値の近傍範囲において測定できることが示された。また、第９図（２）と（３）はＧＯＴとＧＰＴの校正曲線を示し、本測定では、β－ニコチンアミドアデニンジヌクレオチド還元型を用いたＵＶ法により行った。混合液の調整と測定温度は３５℃に保ち、微小タングステン電球から発した光をバンドパスフィルターでピーク波長３４０ｎｍにして測定用流路に照射し、シリコンフォトダイオードで２分間の減衰度を検出して各濃度を測定した。その結果、同様に成人の正常値の近傍範囲において測定できることが示された。
【００２３】
【発明の効果】
本発明により極微量の血液を無痛針により血液分析装置上に導き、その上で血球成分と血清あるいは血漿成分の分離を行った後に、当該血清あるいは血漿成分を種々の試薬と混合し、肝機能を表す指標であるγ－ＧＴＰ、ＧＯＴおよびＧＰＴを比色法によって測定することができることが示された。これにより従来の大型かつ高価な装置を用いて長い検査時間を要したものが、各人が家庭で手軽に短時間で検査できると考えられ、結果として病気の予防に寄与することができる。
【図面の簡単な説明】

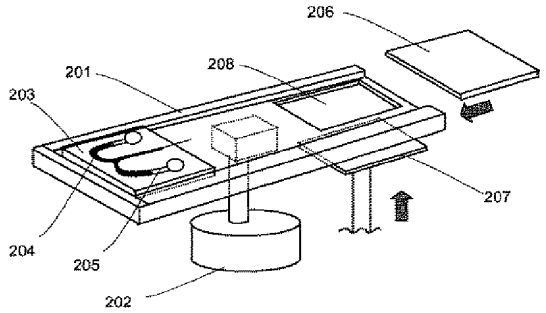
【第１図】無痛針から血液を採取し、血球分離用チップに注入する装置の概略図。
【第２図】オンチップ血球分離法の概略図。
【第３図】γ－ＧＴＰ測定方法の概略図。
【第４図】ＧＯＴとＧＰＴ測定用のチップの概略図。
【第５図】ミキサーの構造と混合方法の概略図。
【第６図】参照用流路と測定用流路の断面の概略図。
【第７図】参照用流路と測定用流路を上から見た概略図。
【第８図】当該マイクロチップの参照と測定用流路の部分の製造プロセスの概略図。
【第９図】（１）γ－ＧＴＰ、（２）ＧＯＴ、（３）ＧＰＴの各校正曲線を示す。
【符号の説明】
（１０１）無痛針
（１０２）円筒管
（１０３）血球分離チップ
（１０４）吸引冶具
（１０５）外部ポンプと接続されるパイプ
（１０６）W字状流路
（１０７）ホルダー
（１０８）血球分離チップの挿入と取り出しを行う冶具
（１０９）バイパス
（２０１）モータと結合した遠心分離用治具
（２０２）モータ
（２０３）W字状流路の血球分離用チップ
（２０４）分離された血球
（２０５）血漿
（２０６）３マーカの各測定用チップ
（２０７）ペルチェ素子
（２０８）開孔部
（３０１）γ－ＧＴＰ測定用チップ
（３０２）モータの軸
（３０３）オンチップ遠心分離用冶具
（３０４）W字状血球分離用チップ
（３０５）無痛針
（３０６）遠心分離後の血球
（３０７）血漿または血清
（３０８）針内での血液詰まりによる引き抜き防止用のバイパス
（３０９）ふた
（３１０）２箇所の取り出し口
（３１１）γ－ＧＴＰ測定用チップの取り入れ口
（３１２）パイプ
（３１３）ミキサー
（３１４）血漿または血清溜め
（３１５）吸入口
（３１６）パイプ
（３１７）液溜め
（３１８）参照用流路
（３１９）引き口
（３２０）測定用流路
（３２１）引き口
（３２２）発色剤溜め
（３２３）高輝度赤色発光ダイオードなどの光源

（３２４）シリコンフォトダイオードなどの光検出器
（４０１）Ｗ字状血球分離用流路
（４０２）取り出し口
（４０３）ＧＯＴまたはＧＰＴの測定用チップの取り入れ口
（４０４）パイプ
（４０５）血漿または血球溜め
（４０６）ミキサー
（４０７）接続パイプ
（４０８）吸入口
（４０９）測定用流路
（４１０）測定混合液の引き込み口
（４１１）バンドパスフィルター付微小タングステンランプ
（４１２）シリコンフォトダイオード
（５０１）ミキサーチップ
（５０２）回転中心
（５０３）血漿または血清の注入口
（５０４）血漿または血清のリザーバ
（５０５）試薬の注入口
（５０６）試薬リザーバ
（５０７）回転方向
（５０８）混合容器（ａ）
（５０９）混合容器（ｂ）
（６０１）光源
（６０２）石英窓
（６０３）流路
（６０４）流路の端
（６０５）通過した光
（６０６）石英窓
（６０７）受光器
（６０８）流路端
（６０９）注入口
（６１０）出口
（７０１）流路
（７０２）曲げ部
（７０３）４５度曲げ部
（７０４）テフロン膜や金属膜
（８０１）金型
（８０２）ポリマー基板
（８０３）フッ素樹脂
（８０４）光の入射口
（８０５）光の出射口
（８０６）石英窓

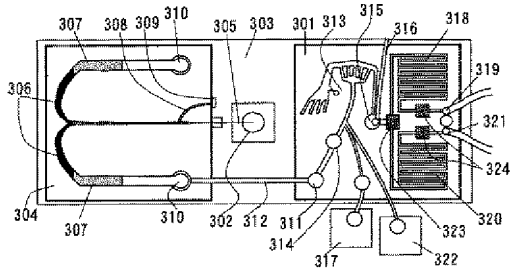
【図１】



【図２】



【図３】



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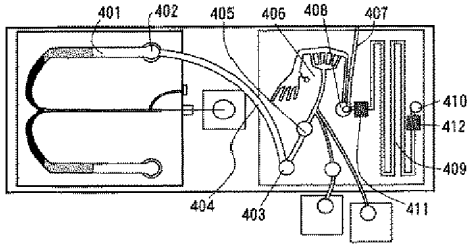

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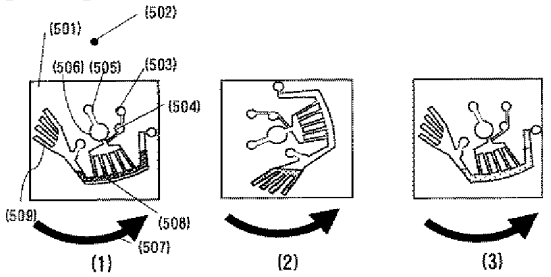

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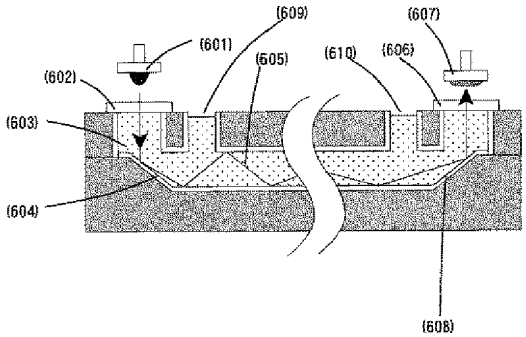
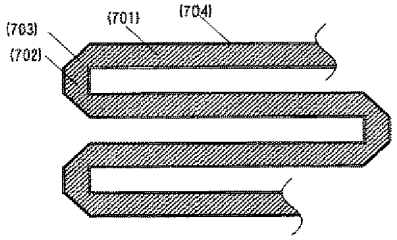
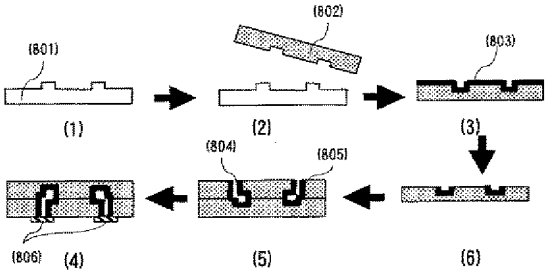


【図７】



【図８】



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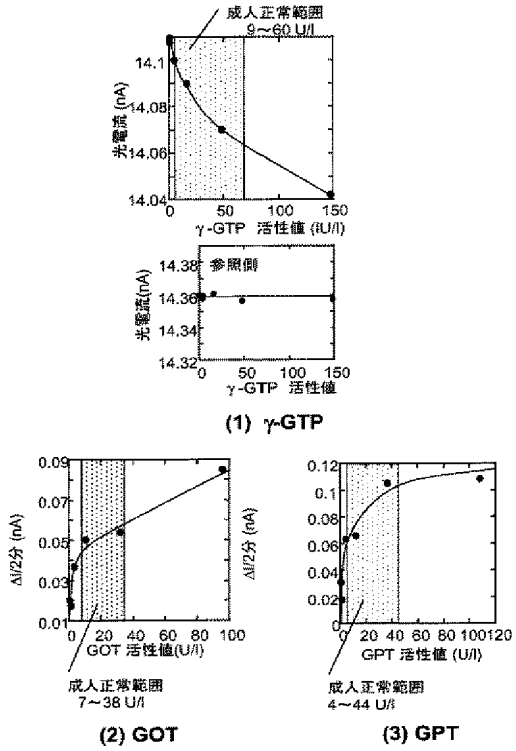
成人正常範囲
9～60 U/l
光電流 (nA)
γ-GTP 活性値 (IU/l)
参照側
光電流(nA)
γ-GTP 活性値
(1) γ-GTP
Δ/2分 (nA)
GOT 活性値(U/l)
成人正常範囲
7～38 U/l
(2) GOT
GPT 活性値 (U/l)
成人正常範囲
4～44 U/l
(3) GPT

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Ｇ０１Ｎ	35/08	Ｇ０１Ｎ	35/02	Ｄ	
// Ｇ０１Ｎ	21/27	Ｇ０１Ｎ	35/08	Ａ	
Ｇ０１Ｎ	37/00	Ｇ０１Ｎ	21/27	Ｚ	
		Ｇ０１Ｎ	37/00	１０１	

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2G057 AA01 AB01 AB03 AB06 AC01 AD02 BA05 CA05 DA03 DA11
DB05 DC06
2G058 AA09 BA06 DA07 FA07 GA06
2G059 AA01 BB04 BB13 DD03 EE01 EE11 FF12 GG02 GG03 GG10
HH02 HH03 HH06 JJ03 KK01 LL03 MM14